4. Title: Microdetermination of Shigella Hemagglutinations in Human Sera

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Period of Report: 1 December 1967—31 March 1968

Objective The finding that Shigellae rather than Salmonellae are likely to cause diarrhea in U.S. personnel in Thailand and that shigellosis is less severe than salmonellosis in Thais stimulated this study to determine the prevalence of shigellosis as measured by antibodies in both populations. The practical importance of this technique lies mainly in its use in making a serodiagnosis of Shigellae infections in epidemiologic studies, particularly in those instances where the patients are no longer excreting organisms, making culture diagnosis impossible.

Description Antigens were prepared by heating phosphate buffered saline suspensions of Shigellae most frequently isolated from U.S. personnel. These were Sh. dysenteriae 1, Sh. flexneri 2, Sh. flexneri 3, Sh. sonnei from I and Sh. sonnei from II. Antisera obtained by immunizing rabbits with these antigens were used for titrations of human and animal erythrocytes in the microtiter system. It was found that sheep erythrocytes were satisfactory and were chosen for routine use. Sera assayed were from diarrhea patients from whom Shigellae had been isolated at least 10 days before sera was obtained, US servicemen in Vietnam who were hospitalized for FUO (fever of undetermined origin) and outpatients at Children's Hospital, Bangkok Thailand who were seen for diseases other than diarrhea.

Progress Median titers obtained with these sera are shown in Table 11. Titers of convalescent sera obtained from 8 diarrhea patients whose stools were positive for Sh. sonnei from 1 indicate there was a hemagglutinative antibody response to infection with this organism. Other Shigellae isolates were too few to permit speculation with reference to antibody response. The finding that the median hemagglutination in Vietnam was low and constant with all antigens suggested that many of the positive antibodies titers of U.S. servicement were nonspecific. The finding of a wide distribution of Shigellae antibodies in the sera of children with diseases other than bacillary dysentery is in keeping with similar findings in Japan and the United States. The gradual increase of titers to all but Sh. dysenteriae 1 could be attributed to greater exposure to these antigens with age.

Hemagglutination titers found in the sera of shigellosis patients were lower than those found in similar studies elsewhere. One possible explanation is that effective antimicrobial therapy blunted the hemagglutinin response. Different techniques for antigen preparation are being studied in an attempt to increase the sensitivity of the test.

Summary Studies have been initiated to determine prevalence of Shigellae hemagglutinins in sera from Thai and U.S. nationals. Convalescent sera from Sh. sonnei patients had relatively high titers to antigens of that organism but not to other Shigellae antigens. Shigellae hemagglutination titers of Thai children appear to increase with age.

Table 1. Enterobacteriaceae Isolated from Acute Diarrhea Cases in Thailand (1 April 1967—31 March 1968)

		Thai	Nationals			American Nationals							
Months	No. of	No. of Patients	Salmonellae	Shigellae	Vibrios	No. of Specimens	No. of Pateints	Salmonellae	Shigeilae	Vibrios			
April 1967	309	158	23	10	_	41	41	-	3				
May	242	133	18	12	1	64	64	_	11				
June	240	108	27	7	_	47	47	_	3				
July	303	157	26	3		28	28	_	1				
August	249	125	8	9	_	28	28		1				
September	156	124	18	5	-	45	45	3	6	_			
October	376	165	32	6	_	38	38		2				
November	481	173	42	14		107	107	_	20	_			
December	453	121	34	6	_	81	81		16	-			
January 1968	382	178	31	6	3	100	100	7	8				
February	269	114	30	8	1	69	69		15				
March	406	153	25	10	2	65	65	1	6	_			
Total	3866	1709	314	96	7	713	713	11	92	_			

Table 2. Salmonellae Isolated from Acute Diarrhea Cases
1 April 1967—31 March 1968

Species		Thai N	lationals			American	Nationals	
Species	Children	Adults	Unknown	Total	Children	Adulte	Unknown	Total
Salmonella paratyphi B	205	_	-	205		_		خبنت
S. derby	24	1		25	_	4		4
S. typhimurium	2	_		2		_		_
S. stanley	7			7	_		_	_
S. montevideo	15			15		_	_	-
S. newport	3			3	_	2		2
S. tananarive	4		-	4	_	_	_	
S. bovismorbificans	1		_	1	_	_	_	_
S. oslo	3.	_		3		_	_	_
S. group D ₂ species	1	_	_	1				_
S. typhosa	8	_	_	8		_		
S. weltevreden	16	_		16	_			
S. lexington	7	1		8			_	
S. anatum	5	1		6				_
S. group D. species	7	_		7		_	_	_
S. newlands	1	-	_	1	_	4	_	4
S. virchow	1		_	1	1		_	1
S. moscow	1		_	1				
Total	311	3	_	314	1	10	_	11

Table 3. Shigellae Isolated from Acute Diarrhea Cases
1 April 1967—31 March 1968

Species		Thai N	lationals			American	Nationals	
Species	Children	Adults	Unknown	Total	Children	Adults	Unknown	Total
Shigella flexneri 1	1.		_	1	1	-		1
Sh. flexneri 2	25	2	_	27	3	10	_	13
Sh. flexneri 3	26	2	_	28	5	4		9
Sh. flexneri 4	_	_	-	_	1	1		2
Sh. flexneri 6	7	_	_	7	2	2		4
Sh. sonnei form I	19	3	-	22	7	49	_	56
Sh. sonnei form II	3	1	_	4	2	_		2
Sh. dysenteriae 1	3		_	3	_	1	_	1
Sh. dysenteriae 2	1		_	1		1	_	1
Sh. dysenteriae 4	1	_	. –	1		_	_	
Sh. boydii 1	1			1		1	_	1
Sh. boydii 2	1			1	_	1		1
Sh. boydii 4	****			_	1			1
Total	88	8		96	22	70		92

Table 4. Sensitivity of Enteric Bacilli to Colimycin (1 April 1967—31 March 1968)

	No. of				Inhib	ited at	mcg/ml				
	tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	139	24	85	23	7						
S. anatum	5	1	4			_		_	_	-	_
S. newport	9	1	3	3	2	_			_	-	_
S. montevideo	6	3	2	1	_			_	_	_	_
S. lexington	10	_	5	3	2	_		_	_	_	_
S. derby	23	5	8	8	_		1	1		_	_
S. weltevreden	15	_	10	5		_			_	-	-
S. stanley	7	4	1	2	_		_		-		_
S. virchow	2		il	ī		_		-		_	~~
S. oslo	3	1		2		_		_		_	_
S. saint paul	6	i	3	ī	1				_	. —	_
S. typhimurium	11	3	5	2	i	_	_ [_		-	_
S. typhoso	9	3	2	1		2	1		_	_	_
S. panama	1 1		ī		_	_	Į	/ _	_	_	_
S. enteritidis	1 1	[i	_	_	_ [_	_	
S. tananarive	3		i	2	_		/ _	_		_	
S. tennessee	1 1		ı l	_ [_		i	_	_		
S. newlands	1	_	i	-	_		_	_	_	_	
Shigella sonnei form 1	75	38	21	12	1	3	_	_	_	_	
Sh. sonnei form II	4	2	1	_ {	1	[·	_	_ :	_	
Sh. dysenteriae 1	4	3	1	-]	_		_	_]		
Sh. dysenteriae 2	3	2	_ [1	· _			_ [_]	_	_
Sh. flexneri 1	2	2	- 1		_	_ [_	_	_ [
Sh. flexneri 2	47	34	12	1		_			_		
Sh. flexneri 3	47	37	7		1	1		_	_ [
Sh. flexneri 4	7	5	2	_		_		·	_		
Sh. flexneri 6	8	7	1	- 1	_ l	_]	_ 1	_ [
Sh. boydii 2	3	3	-	_	_	_	_ [_]	_	_	_
Sh. boydii 4	4	4	-		-			-		_	-
Escherichia coli 055:B5	6	5	_	_		_	_	_]	,	_	_
E. coli 026:B6	9	7	1	. —	1	_ i	[[_ 1	_	_
E. coli 086:B7	3		1	1	1]	1	_ [_	_	
E. coli 0127:B8	23	7	6	7	3	_	_		_		_
E. coli 0112:B11	6	3	2	1		1	_	_]	_ [_
E. coli 0128:B12	31	8	14	1	6	_	2	_	_ i		
E. coli 0119:B14	88	43	11	12	5	5	_ [6	- 6	_ [
E. coli 0125:B15	35	22	6	2	5	_	_ [_	_ [_ [
E. coli 0126:B16	23	18	2	1	2	_	-	_	_ 1	_	_
E. coli 0124:B17	2	1	_	_	1	_	_	_ [_		_
E. coli 025:B19:B23	85	49	22	7	6	1				_	_

Table 5. Sensitivity of Enteric Bacilli to Nalidixie Acid (1 April 1967—31 March 1968)

	No. of				Inhibit	ed at m	ncg/ml				
	strains tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	92	5	6	21	21	35	3		_	_	1
S. anatum	3			1		2		_	_		
S. newport	8	2	1	2	_	2	1				_
S. montevideo	2	_			1		1		_		
S. lexington	6	_	_	2	4		_			_	_
S. derby	15		2	8	ī	4			_		_
S. weltevreden	7	1	_	_	6			_	:	_	_
S. stanley	3	1 1	_	_	1	1			_	_	l _
S. virchow	2	_	l –	1 1	l –	1	_		l _	l _	l _
S. oslo	1			_	1		_		l _	l _	l _
S. saint paul	6		_	1 1	3	2	l _	_		_	l _
S. typhimurium	10] 1	_	4	3	2		_	_	<u> </u>	l _
S. typhosa	5	1	2	2	_	'	_	_		_	i _
S. panama	1	_	1				_	_		_	
S. enteritidis	1 1	_	1	_		_		_	_		
S. tananarive	1	-	-	1	-	_	_	_	-	-	_
Shigella sonnei form I	50	15	4	18	7	6	_	_	_	_	
Sh. sonnei form II	4	3	_	_	1	_	_		l _		-
Sh. dysenteriae 1	4	2	2		1 _		l _	_	l _	_	-
Sh. dysenteriae 2	2	1	_	1	_	l _		_	!	_	-
Sh. flexneri 1	1		l –	1	1	_	_	l _	_	_	1 _
Sh. flexneri 2	27	12	9	1	3	2	l _	_	l _		
Sh. flexneri 3	37	17	8	1 4	6	2	I _	_	_		1 _
Sh. flexneri 4	6	3		1 _	1 1	2	_	_		i _	
Sh. flexneri 6	3	3	_	_		1 _	_		_		_
Sh. boydii 2	1	1 1		1 _		_] _	l _			
Sh. boydii 4	2	2	_	-	-	-	-	-	_	-	-
Escherichia coli 055:B5	6	2	1	_	1	2	_	_		_	_
E. coli 026:B6	8	4	_	2	1	ī			l _	_	_
E. coli 086:B7	2	1	1	_	_	_	_	_	_	-	_
E. coli 0127:B8	18	6	1	3	7	1	_	_	-	-	-
E. coli 0112:B11	1	-	-	_	-	1	-	-		1 -	-
E. coli 0128:B12	28	4	2	6	9	4	3	-		1 -	-
E. coli 0119:B14	57	12	12	12	15	3	1		2	-	-
E. coli 0125:B15	24	3	5	7	5	4		-	-	-	-
E. coli 0126:B16	19	8	2	3	2	-	2	-	-	1 -	2
E. coli 0124:B17	2	1	-	-	1	-	-	-	-	-	1 -
E. coli 025:B19:B23	57	23	14	5	6	7	1	I -	1 -	-	1

Table 6. Sensitivity of Enteric Bacilli to Neomycin (1 April 1967—31 March 1968)

	No. of				Inhibit	ted at	mcg/mi				
	tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	140	1			3					_	
S. anatum	5		1 1	_	1		-	l -	-	1	135
S. newport	9			1	-	1	1	1	-	_	–
S. montevideo	6	_	-	' '	3	1	1	1	-	1	1
S. lexington	10	-	1	_	_	4	1	_	_	_	-
S. derby	1	_	3	4	2	1	_	-			
S. weltevreden	23	_	5	12	6			-	-	_	_
S. stanley	15	_	3	7	3		2	-			_
S. virchow	7	-	3	2	2	-	-	-	_	_	l –
	2	_	-	1	_	1 1		_	_		_
S. oslo	3		1	2	_	_	l		_		
S. saint paul	6	_	2	4	_		l _	_			_
S. typhimurium	11	_	1	6	4		l _		_		_
S. typhosa	9	2	3	3	1	_		_	_		
S. panama	1 1			_	1	_	_	_		_	_
S. enteritidis	1	_	_		i				_		_
S. tananarive	3	_				- 1		_	-		1
S. tennessee	1		1	1	-			_		1	ı
S. newlands	i	_	_	i	_	-	_	_	_	_	_
Shigella sonnei form l	75		1	28	27	,				;	
Sh. sonnei form II	4	-			37	7	2	-			_
Sh. dysenteriae 1	8	-	_	1	_	1	1	_	_		1
Sh. dysenteriae 2	4	_	2	1	1	_	_				-
Sh. flexneri 1	3	_	1	_	1		-	_	-	_	1
Sh. flexneri 2	2	_	-	_	-	2					2
Sh. flexneri 3	46	-	1	6	15	21	1			_	2
Sh. flexneri 4	47	-	1	10	25	11		_	_		_
	7		_	1	6		_	_	_	_	_
Sh. flexneri 6	8		-	5	2	_			1		_
Sh. boydii 2	3		1	1	1			_			_
Sh. boydii 4	4		_		2	2	-		_	_	
Escherichia coli 055:B5	6	1	1		1	1		_		ı	1
E. coli 026:B6	9	1	2	3				-		•	3
E. coli 086:B7	3	_		2			_			_	1
E. coli 0127:B8	20		1	2	2	_	_	_	-	_	
E. coli 0112:B11	5	I —	,	4		۱ ۱	1	_	_	I	12
E. coli 0128:B12	32			_	1	-	_	-	_		3
E. coli 0119:B14	85	3	1	3	7	5	1	_	_	2	13
E. coli 0125:B15		l ³	4	3	4	5	_	1	3	4	58
E. coli 0126:B16	33	-	3	4	4		2	_	2	2	16
E. coli 0124:B17	23	-	2	1 1	_		_		_	_	20
E adi 025 210 200	2	_	-	1	-	1	_		-	_	_
E. coli 025:B19:B23	84	1	1	7	9	1	1	_		1	63

Table 7. Sensitivity of Enteric Bacilli to Furazolidone (1 April 1967-31 March 1968)

	No. of				Inhibit	ed at n	ncg/ml				
	strains tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	85	1	1		1	2	24	44	6	4	2
S. anatum	1	1	_	<u> </u>		_	_	_		_	_
S. newport	2		2	_							_
S. montevideo	1		—	l –	-	_	1	-	l _		_
S. lexington	5	3	- 2	-		_ '	_				_
S. derby	11	7	1	3			_	_		_	_
S. weltevreden	9	7		1	1	_	<u> </u>]	_	l	l
S. stanley	2		2	 	_				_	1 _	
S. virchow	1 1	1	l	_	_					l _	
S. oslo	2			1	1			-		l _	l _
S. typhosa	2	_	_	2				_	l _	l _	l _
S. tananarive	1	1	_	_	_	l –	_		_		_
S. tennessee	1	1	_			-	_	_	_	_	-
Shigella sonnei form l	28	13	8	4	2	1			_		_
Sh. sonnei form II	2	1 1	_	1 1	l		_		_	<u> </u>	
Sh. dysenteriae 1	1 1	1				_		l		<u> </u>	l _
Sh. flexneri 1	2	2		_	l		<u> </u>		l _	_	_
Sh. flexneri 2	19	9	7	3	<u> </u>	<u> </u>		_		l _	
Sh. flexneri 3	16	3	5	6		2	_		1 _	l	
Sh. flexneri 4	3	2	1 –	_	1		_	_		_	
Sh. flexneri 6	1 1	1 1	_	_					l _		_
Sh. boydii 4	1	-		1	-	-	-	_	-		-
Escherichia coli 055:B5	4	_	1	1	_	1	1			_	_
E. coli 026:B6	7	1	li	2	1 1	1	1	l _	1 _		
E. coli 086:B7	2	1 1	 	1	_		l _	_		<u> </u>	 -
E. coli 0127:B8	8	1 1	3	1	3	_	_	_	_		
E. coli 0112:B11	2		_	2		_	_	_	_	1 _	1 _
E. coli 0128:B12	12	5	1	3	1	2		_		_	_
E. coli 0119:B14	50	3	12	12	14	5	2	2	_	l –	_
E. coli 0125:B15	10	li	4	5	1 _		_		_	_	
E. coli 0126:B16	5	2	2		I _	1				_	1 –
E. coli 025:B19:B23	38	10	6	6] 3	5	8	l _		_	_

Table 8. Sensitivity of Enteric Bacilli to Chloramphenicol (1 April 1967-31 March 1968)

	No. of strains				Inhibit	ed at	mcg/ml				
	tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	140	3	3	1	1						
S. anatum	5	1	_	4			-		6	40	86
S. newport	9	2	2	4	1	_	-	-	-	-	-
S. montevideo	6		_	1		_	_	-	-	-	-
S. lexington	10	1	1	3	-	_	-	-	-]	5
S. derby	23	2	4	13	1		1	-	1	-	2
S. weltevreden	15	2	1	10	4	. —	-	-	-		-
S. stanley	7		2	4	1		-	-	1	-	_
S. virchow	2			2	1	_	<u> </u>	-	_	-	-
S. oslo	3	_	_	3	_	-	-	-	-		-
S. saint paul	6	_ 1	_		-	_	-	_	-	-	i
S. typhimurium	11	3	5	-	_		_	-	-	-	5
S. typhosa	9	5	3	3		_	. —	-	_	-	-
S. panama	1		1	- 1	- 1	_	-	-	-		1
S. enteritidis		_	;	- 1	-		·	-		_	_
S. tananarive	3	-	- 1	-	- [-		-			_
S. tennessee	, ,	- 1	1	1	<u> </u>			_	- 1	_	1
S. newlands	1 1		-	-	1 1	-	_	_		_	l –
·	1 1	-	1	-				-			. –
Shigella sonnei form I	75	2	3	_ , [1						
Sh. sonnei form	4	i	_	4	1	1	_	3	4	16	41
Sh. dysenteriae 1	4	-		'	-		_	_		2	1
Sh. dysenteriae 2	3	- 1	1	_ [-	_	-	3		1	
Sh. flexneri 1	2		· .	-		-		_	1	-	_
Sh. flexneri 2	47	- 1	-	- 1	-	-	_	1	1	-	-
Sh. flexneri 3	47	_		1	-		3	20	20	2	-
Sh. flexneri 4	7				- 1	- 1	3	3	27	. 8	5
Sh. flexneri 6	8	1	-	-	_	- 1	1	- 1	3	2	_
Sh. boydii 2	3		-	1	- 1	-	-	2	2	2	_
Sh. boydii 4	4	-	1 2	-	-	- 1	_	-	2		
	1 1	'	4	-	-	_		1		-	
E. coli 005:B5	6	_	2	l	1	[_	
E. coli 026:B6	8			1	2	-	-		-	2	1
. coli 086:B7	3	_	ī	' [. 4	-	1	_	2	_	2
. coli 0127:B8	23		'	2	3	-	- 1		1	1	_
. coli 0112:B11	6	_	_	2	2	1	1		1	3	13
. coli 0128:B12	34	3	1	3	'	- 1	-	-	_	1	3
. coli 0119:B14	91	٠			- 1	-	- 1	1 [3	12	11
coli 0125:B15	35	-	3	4	3	-	1	4	14	29	33
. coli 0126:B16	21	_	6	4	2	-	4	1	8	4	6
. coli 0124:B17	2 2	- 1	-	3	-]	-	1	10	1	2	4
. coli 025:B19:B23	84	_	-	2	- 1	-	- 1	- 1			****
023:014:052	1 54	1	1	4	2	4	-	3	26	29	14

Table 9. Sensitivity of Enteric Bacilli to Oxytetracycline (1 April 1967-31 March 1968)

	No. of				Inhibit	ed at n	ncg/ml	,			
	strains tested	0.78	1.56	3.12	6.25	12.5	25	50	100	200	> 200
Salmonella paratyphi B	139	2	1	1		1	_		6	51	77
S. anatum	5	2	1	1	_		1		_	_	
S. newport	9	_	5	1	I –	2	_	1		_	_
S. montevideo	6	l _	1	3	2	_	_		<u> </u>		
S. lexington	10	l _	5	l _	_	1	i	_	_	_	4
S. derby	23	2	18	3	_]	l –	_	_
S. weltevreden	15	1 4	6	2	2	1] _	l	_
S. stanley	7	2	2	1		1	<u> </u>	1	l –	I –	-
S. virchow	2		2				l _	_	_	_	-
S. oslo	3	1 1	2	_			_		_	_	l –
S. saint paul	6	1	1 1	1 _	_	3	1	1	l _	_	_
S. typhimurium	11		4	1		6			 	_	_
S. typhosa	9	2	5		1 1	l i	l _	_			_
S. panama	i		_		l i	<u> </u>	l _	_	l _		· —
S. enteritidis	1	l .		1 _	i	_		_		l _	_
S. tananarive	3		2		<u> </u>	l _	_	l _		<u> </u>	1
S. tennessee	1	1	l î		l _			_	_		_
S. newlands	li	_	li	_	-	_	_		_		_
Shigella sonnei form I	75	2	2	4	2	2	40	18	_	1	4
Sh. sonnei form li	4		1	_		_	2		_	1	-
Sh. dysenteriae 1	4	_	1	_	1 –		_	_	1	2	-
Sh. dysenteriae 2	3	1 1	_	_	1	_	I –	-		-	1
Sh. flexneri 1	2		_	_	_	_	_	_	1	1	-
Sh. flexneri 2	47	1		2		12	23	7	1	1	-
Sh. flexneri 3	42	<u> </u>	1 _	1	l -	1	2	7	8	15	8
Sh. flexneri 4	7	1		_	_	1 1	2	1	l	2	1
Sh. flexneri 6	8	1	I _	_	_	1	_	2	2	2	-
Sh. boydii 2	3		_	1 1	_		1	_		_	1
Sh. boydii 4	4	-	4	-	-	-	-	-	_	-	-
Escherichia coli 055:B5	6	_	_	1	1	_	_	_	_	2	2
E. coli 026:B6	9	_	1	1	1	-	-	-	-	2	4
E. coli 086:B7	3	_		1	-	_	_	-	_	-	2
E. coli 0127:B8	23	_	1	2	1	_	-	1	2	3	13
E. coli 0112:B11	6	_	_	_	_		-	2	-	-	4
E. coli 0128:B12	34	_	_	_	1	1	-	2	2	11	17
E. coli 0119:B14	90	_	4	5	2	-	2	8	3	17	49
E. coli 0125:B15	35	1	4	_	_	1	2	5	1	6	15
E. coli 0126:B16	23	1 1	_	_	_		1	_	1	6	14
E. coli 0124:B17	2		I -	1 1	1	_		_	1 —	_	-
E. coli 025:B19:B23	84	1	8	1 1	2		I –	5	4	15	48

Table 10. Rank Order* of Viable Counts of Fecal Bacteria in Stools of Thai Infants

	Rank	order
Organism	Acute specimens (34)	Convalescent specimens (14)
Coliforms	1	2
Gram negative anaerobic bacilli	2	
Alpha—hemolytic streptococci	3	5
Lactobacilli	4	3
Yeasts	5	6
Streptococcus fecalis	6	4
Staphylococci	7	7

^{*} Highest counts, 1; lowest count 7

Table 11. Median Hemagglutination Titers of Sera with Sheep Erythrocytes Sensitized with Shigellae Antigens

	No. of	Shigella Antigen								
Source of Sera	Sera	Sh. dysenteriae 1	Sh. sonnei l	Sh. sonnei II	Sh. flexneri 2	Sh. flexneri 3				
Shigella sonnei from I Patients	8	16	128	8	16	8				
Other Shigellosis Patients	6	8	8	8	16	32				
J.S. Servicemen in Viet Nam (FUO)	102	8	8	8	8	32 8				
hai Children Outpatients		_			ľ	•				
(Non-diarrheal)										
<1 yr	17	8	16	8	8	8				
1—3 yrs	65	16	32	16	32	16				
3-5 yrs	33	8	32	32	32	32				